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Environmental Assessment of a Phthalate Ester DI-(2-Ethyl Hexyl) Phthalate (DEHP) Derived from a Marine Microcosm

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16. ABSTRACT

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ABSTRACT

This study tested the influence of system complexity on the environmental assessment of a chemical. Marine microcosms were perturbed with a phthalate ester, di-2-ethyl hexyl phthalate (DEHP).

Concentrations of 1, 10, and 100 ug DEHP/l were added to the water column subsystem of marine microcosms for 30 days during the summer (18°C) and winter (1°C) seasons. Significant portions of the DEHP losses from the water column subsystem during the experiments were due to the complete breakdown of the parent compound to CO_2. In the benthic subsystem, DEHP concentrations in the sediment were increased relative to the water column by over 50 and 250 times in the winter and summer seasons, respectively. Statistically significant (α'=0.05) reductions in the fluxes of NH_3 from the benthic subsystem were observed in microcosms receiving 100 ug DEHP/l during the summer. Concentrations of DEHP in selected bivalves ranged from 174 - 229,000 ug DEHP/wet kg depending upon the water column exposure concentrations and the season of the year.

Key Words: complexity, temporal heterogeneity, seasonality, microcosm, DEHP, phthalate

INTRODUCTION

Fate and ecological effects are necessary elements for an environmental assessment of a chemical. Such data are usually derived indirectly from experimental laboratory systems. The biotic complexity of most of these systems is relatively simple, i.e., they consist of one species. Furthermore, the seasonal temperature at which these studies are performed is usually at the elevated portions of an annual cycle, 20-25° C. The question arises whether the simulation of biotic complexity and temporal/seasonal heterogeneity have any significant influence on the fate and ecological effects of a chemical and, therefore, its environmental assessment.

According to the National Research Council (1), "Single species tests,... do not provide an accurate estimate of either a species response (to a chemical) in the natural environment or indirect effects that might occur in other components of a (eco) system (pg. 77)." However, experiments testing the influence of biotic complexity and temporal heterogeneity on the fate and ecological effects of a chemical are extremely rare especially in marine ecosystems. As a result, this study attempted to determine the significance of laboratory simulation complexity on the environmental assessment of a chemical in marine ecosystems. Specifically, a biotically complex microcosm simulating a north temperate marine ecosystem was perturbed with di-2-ethyl hexyl phthalate (DEHP) during two major time periods, the winter and summer seasons. DEHP was chosen as the perturbant because it is an input into the marine environment as evidenced by its wide distribution (2).

MATERIALS AND METHODS

The ecological marine system simulated in the laboratory was a portion of Narragansett Bay, Rhode Island, USA. This system is composed of a water column subsystem coupled to a sediment or benthic subsystem. The water column exchanges with both the ocean and the upper reaches of Narragansett Bay. Seasonal temperature conditions in the water column range from -2 to 28°C.

The experimental microcosms used in this study (Figure 1) simulated the major physical characteristics of the natural system as well as the major biotic communities found in the water column and benthic subsystems. The microcosms were 140 litre tanks containing seawater and a benthic box core, both collected from the natural system being simulated. Conditions of temperature, light (intensity and photoperiod), turbulence, ratio of benthic surface area to water volume, and water turnover were adjusted to approximate conditions measured and/or expected in the natural system. All components of the entire microcosm system were made of hard glass to minimize adsorption of DEHP. (see (3) for the rationale of microcosm system). Water turnover (exchange) in the microcosm tanks was simulated by the removal, and replacement with bay water of 10 litres of water per tank three times a week.

Two experiments were performed. Each consisted of four treatment (DEHP exposure) conditions with five replicates per treatment. One experiment was performed during the summer (average temp. = 18°C) and the other during the winter (average temp. = 1°C). The desired initial exposure concentrations in the water column were 1, 10, and 100 ug DEHP/l.

Concentrated stock solutions of DEHP were made up in an acetone carrier with DEHP-C-14 (American Radiochemical Corporation) and DEHP-C-12 (Aldrich Chemical Company). These concentrations were based upon the

manufacturers specific activities. However, the stated specific activities were found to be incorrect when analyzed by high pressure liquid chromatography (HPLC) and liquid scintillation radio-spectrometry (LSC). Because of this and other factors, the resulting concentrations of DEHP at the start of the winter experiment were 1.01, 11.01, 101.1 and 1.04, 8.18, 98.18 ug/l in the summer experiment. The quantitative significance of these differences were tested by comparing the original data sets to identical data sets adjusted for the discrepancies between the desired and the actual input concentrations. The adjustment procedure consisted of multiplying all individual data by the ratio of the desired to actual input concentration.

The DEHP was homogenously added to each microcosm in the following manner. An appropriate concentraion of stock solution was pipetted into one litre of seawater contained in a two litre separatory funnel. The funnel was shaken vigorously for two minutes before addition to the stirred microcosm a few centimeters below the air-water interface. The separatory funnel was rinsed with 10 ml of acetone and one litre of seawater, shaken for an additional two minutes and added to the microcosm. DEHP was added to the microcosms in the same manner during the subsequent water exchanges, but only in quantities sufficient to achieve the original test concentration in the 10 litres of replacement water. This was done over the duration of the 30 day experiment at a rate of three times per week.

After the initial addition of DEHP to the microcosm, seawater samples for DEHP were taken at 1,2,4,8, and 24 hours. Seawater (DEHP) samples were subsequently collected just prior to each water exchange over the duration of the summer and winter experiments.

Water samples were collected through a five cm (diameter) open-ended cylinder which was suspended from the side of the microcosm tanks and extended approximately 10 cm below the air-water interface. Floating a cellulose acetate filter pad, for approximately one minute, on the water surface within the cylinder, effectively removed the DEHP surface film and allowed the collection of water samples free of film concentration.

We found that DEHP could be quantitatively removed from seawater by filtering through 0.22 micron cellulose acetate filters. Consequently, replicate ten millilitre water samples for DEHP were filtered through 0.22 u, 25 mm cellulose acetate filters supported on a stainless steel filter support. The filter pads were placed into scintillation vials and dissolved in 20 ml of a scintillation cocktail prior to counting for ten minutes.

Since DEHP was found to be degraded to more polar intermediates and even to carbon dioxide C-14 which could be re-incorporated back into the biological food chains, it was necessary to distinguish DEHP-C-14 from the total C-14 activity in all portions of the microcosm sampled. The percentage of DEHP-C-14 to total C-14 in water was determined by filtering 100 to 1000 ml seawater onto glass fiber filters (GFF). The GFF were first eluted with methanol and then pentane. The pentane was partitioned from the methanol by the addition of water and shaking in a separatory funnel. The pentane fraction which contained the DEHP was separated by HPLC and quantified by LSC. In the winter, greater than 95% of the total activity on the filter pad was DEHP-C-14; whereas in the summer, approximately 80% was found to be DEHP-C-14.

Water samples for carbon dioxide ($^{14}CO_2$) analysis were collected weekly by pipetting 100 ml of seawater in gas sparging tubes. The samples were acidified with HCl, sparged with helium and the $^{14}CO_2$ was trapped in an organic base (4). The levels of $^{14}CO_2$ were determined by LSC.

At the end of the experiment three to five cores were removed from the benthic box core with modified 50 ml plastic syringes and frozen. The frozen cores were sectioned into 0-1, 1-2, 2-3,3-5, and 5-7 cm sections in order to obtain a DEHP concentration depth profile. Each fraction was extracted with methanol and an aliquot counted for radioactivity. The 0-7 cm methanol extracts were combined and extracted against pentane. Aliquots of the concentrated pentane fraction were separated by HPLC and quantified by LSC to confirm the DEHP concentrations. After methanol extractions, all sediments were dried, combusted and any residual C-14 activity was released as $^{14}CO_2$ and trapped in an organic base for liquid scintillation counting (5). The remaining benthic substrate was screened (0.5mm), the organisms identified, counted, and selected species prepared for chemical analysis. The zooplankton from two tanks from each treatment were also collected at the conclusion of the 30 day experiment.

The body burdens for the bivalves, Pitar morrhuana and Nucula annulata and the summer values for the polychaete, Nepthys incisa were determined by direct analysis of DEHP. This was accomplished by homogenizing the samples in methanol, centrifuging and then removing an aliquot of the supernant for total radioactivity. The remainder of the methanol supernant was extracted against pentane and seawater. The pentane fraction was separated by HPLC and quantified by (LSC) to determine the actual concentration of DEHP from the total radioactivity.

The body burdens for the zooplankters Acartia clausii and A. tonsa, the bivalve Mulinia lateralis and the winter Nepthys incisa were calculated from the total measured radioactivity in the sample and the specific activity of the DEHP added to the microcosm. The total radioactivity was determined by dissolving the whole animal in a strong organic base, adding scintillation

fluid and then counting the total tissue radioactivity. Winter N. incisa values were adjusted by multiplying the measured concentration by the percent of DEHP to total radioactivity determined for summer N. incisa. M. lateralis body burdens were adjusted based upon the percentage of the total radioactivity that was DEHP as measured in P. morrhuana. The latter organism was chosen because M. lateralis showed identical relationships of DEHP accumulation with respect to season and DEHP inputs (refer to Results).

All wet tissue whole animals were weighed before samples were placed into methanol and homogenized. The wet tissue weights for the two small bivalves, M. lateralis and N. annulata were computed by subtracting the shell weights, previously determined by regressing shell width against shell weight, from the whole animal wet weight.

The methods and sampling frequency for all ecological effects parameters measured in this study were identical to those described previously (3) except bacteria (6) which were measured twice per week and benthic respiration which was not measured.

Transformations of the data were applied where appropriate.

Factorial (4x2) analysis of variance procedures (7) were used to test for variable differences due to the main effects of input concentration and season and the interaction between concentration and season. These procedures were applied to both the original and adjusted data sets. The interaction term should be assumed to be non-significant unless stated otherwise. The significance level was set at α=0.05. However, this factorial analysis procedure could not be applied to the body burden data of P. morrhuana. This was due to the lack of individuals in the summer, 100 ug DEHP/l exposure microcosms. As a result, a one way analysis of variance procedure was used to test for significant seasonal input concentration effects.

The coefficients to the exponential decline of DEHP in the water column were derived via a non-linear statistical program. The average exposure concentration in the water column was then computed by integrating the exponential function for each replicate microcosm over 30 days, the duration of the experiments.

RESULTS

Examination of the body burden and distributional data for DEHP revealed a dependent relationship between the means and associated variances. As a result, it was not appropriate to run an analysis of the data until the means and variances were independent of each other (8). Transformation of these data to Log_{10} was successful in satisfying the independence assumption.

When the analysis of the data adjusted for discrepancies between desired and actual input concentrations was compared to the analysis of the unadjusted data, no differences were found. Therefore, the results presented herein are based upon the analyses of the unadjusted or "raw" data.

DEHP concentrations decreased exponentially from the water column with time after initial additions but at seasonally-dependent rates (Figure 2). Such seasonally-dependent losses were due to the breakdown of DEHP as evidenced by the inverse relationship of a major breakdown product $^{14}CO_2$ (Figure 3). As a result, the average exposure concentrations realized in the water column during the winter season were almost four times greater than that realized in the summer season (Table 1).

The exposure levels of DEHP in the sediment subsystem were dependent upon the initial water column concentrations of DEHP only, since the sediment concentrations of DEHP during the winter and summer seasons were not significantly different from one another. Concentrations of DEHP in the sediment averaged over 0-7 cm, were over two orders of magnitude greater than the overlying water.

Accumulations of DEHP in all the organisms examined were found to be dependent upon the initial water column concentration of DEHP and

season, except for A. clausii and A. tonsa which showed no significant seasonal effect (Table 1).

P. morrhuana and M. lateralis, both bivalve filter feeders (9) had ten times more DEHP in their tissues during the winter experiment. Such a seasonal difference may be due to the higher (a) amount of DEHP in the water column (Figure 1, Table 1) as indicated above and (b) algal densities during the winter experiment (Table 2).

However, the polychaete N. incisa and the bivalve N. annulata, both selective deposit feeders (9), had 5 and 4 times higher body burdens in the summer experiment, respectively (Table 1). Since exposures to DEHP in the sediment were equal in the winter and summer experiments (Table 1), the higher body burdens of N. incisa and N. annulata during the summer experiment may have been due to elevated feeding rates caused by the higher ambient temperatures.

The concentrations of DEHP in the water column during both seasons had no significant ecological effects on any of the water column organisms and ammonia levels (Table 2). However, significant reductions in the flux of NH_3 from the sediment were observed in microcosms receiving initial water column exposures of 100 ug DEHP/l during the summer season only (Table 2). This response was functional since the reduced NH_3 flux was not associated with a corresponding numerical reduction in the densities of benthic species (Table 2).

DISCUSSION

Environmental assessment of DEHP was based upon the potential for (a) human exposures and (b) ecological effects realized within a north temperate marine ecosystem. Human exposures through a man-based food chain require first, identification of the food species through which DEHP can pass, and second, determination of the same species maximum body burdens relative to the DEHP input concentration. In this ecosystem, two pathways were possible, one through the hard shell clam, Mercenaria mercenaria and the other through large crustaceans and fishes. While these specific organisms were not present in our microcosms, data derived in this and previous studies do enable us to draw inferences, with varying degrees of confidence, about the bioaccumulation of DEHP in M. mercenaria if it were present.

P. morrhuana, a hard shell clam present in our microcosms, is in the same family as M. mercenaria and is found together with M. mercenaria under natural conditions. Furthermore, P. morrhuana is considered to be a water column filter feeder (9), as is M. mercenaria. As a result, of these similarities, we would expect the bioaccumulation of DEHP by M. mercenaria to be similar to that of P. morrhuana if exposed to identical water column conditions. Bioaccumulations of DEHP in P. morrhuana were dependent upon the DEHP input concentration to the water column and season. The higher bioaccumulations in the winter season could be due to either the (a) higher water column exposures (Table 1), (b) higher algal densities (Table 2), which adsorbed DEHP from the water column, (c) lower degradation rate by P. morrhuana or (d) various combinations of (a), (b), and (c). In any event, we found that fixed water inputs of DEHP during two extremes of a full seasonal cycle resulted in as much as 10 times

higher bioaccumulation in P. morrhuana during the winter season as compared to the summer. If the bioaccumulation of DEHP in M. mercenaria is similar to P. morrhuana, then the highest human exposure is expected to occur during the winter season and/or when the phytoplankton population is elevated. In this study, concentrations of 25,707 ppb were observed in P. morrhuana during the winter with a fixed DEHP input concentration of 100 ug/l. However, it should be noted that similar inputs of DEHP to a natural system are expected to result in even higher bioaccumulations in P. morrhuana and M. mercenaria. We expect this to occur because a significant fraction of the losses of DEHP from the water column of our microcosms were due to uptake onto glass surfaces (not presented). Such reduced exposures would not occur under natural conditions.

The other potential pathway for human exposure to DEHP identified in this system is through large crustaceans and fishes. These organisms can accumulate DEHP directly from the water column and indirectly through various food species contained within our microcosms. Since we do not know the accumulation of DEHP by either process, it is not possible to predict the concentration of DEHP in the edible portions of any of the known marine crustaceans and fishes under natural conditions. However, it is interesting to note the large differences in concentration existing between the various food species of macrofauna and the water column. Specifically, all the species presented in Table 1 except P. morrhuana have been found in the stomachs of winter flounder (Pseudopleuronectes americanus), a flatfish, collected from the field (10). Concentration differences between M. lateralis, a dietary input of the winter flounder and the water column was almost 4,000 fold during the winter experiment. Future studies need to expose winter flounder and

other macrofauna to not only the water column concentrations of DEHP used in this study, but also the diverse organisms upon which these macrofauna feed.

Ecological effects were observed only in the sediment subsystem where the concentration of DEHP was almost 400 times the level in the water column subsystem during the summer season. Reductions in sediment NH_3 flux occurred when the microcosm input of DEHP was 100 ug/l. Such an input resulted in 6,166 ppb in the upper sediment layers (0-7 cm) at the end of 30 days. However, sediment DEHP decreased exponentially with depth (not shown). This is significant because animals are not uniformly distributed with depth. Rather, between 75% (9) and 89% (11) of the benthic animals are found in the top 2-3 cm of sandy and silt-clay sediments, respectively. As a result, the sediment exposure concentrations that caused the reduced NH_3 flux were three times higher than the concentrations averaged over 0-7 cm as presented in Table 1.

Water column effects due to the reduction in sediment NH_3 flux and possibly other materials were not observed over the duration of the experiment. Long term studies are needed to establish if (a) the observed 30 day functional response at 100 ppb water inputs is a prelude to a numerical reduction in benthic biomass and/or composition and (b) the water column community is effected by the reduced sediment NH_3 flux rates.

Most environmental assessment studies of chemicals in aquatic environments are based upon experimental systems composed of only a water column and a few associated organisms. Furthermore, such studies are most often performed at temperatures which approximate the warmest periods of an annual cycle. If we had developed an environmental assessment of DEHP based upon a microcosm consisting of a water column subsystem held

at summer temperatures, then we would have underestimated the potential hazards of this chemical. We would have observed a rapid and large loss (up to 85% of the total)of DEHP from the water column.. A significant portion of these losses would be associated with the degradation of DEHP to CO_2. The only potential human exposure would be indirect, perhaps through the consumption of fish feeding upon the water column zooplankters, such as A. tonsa Ecological effects of DEHP would not be observed.

In this study the incorporation of a benthos to the water column subsystem along with the addition of DEHP during winter as well as summer seasons results in additional information which significantly changed the environmental assessment of DEHP. First, the inclusion of a benthos resulted in two additional routes of human exposure. One indirectly through fish and large crustacea feeding upon benthic organisms and the other directly through the hard shell clam, M. mercenaria. Second, identical inputs of DEHP during the winter and summer seasons resulted in maximum body burdens in the hard shell clam P. morrhuana in the winter and not the expected summer season. Third, a concentration and seasonally dependent ecological effect, namely a reduced benthic NH_3 flux was observed. Based upon these results, we feel that future environmental assessments of chemicals for north temperate marine and possibly lacustrine ecosystems should include data bases derived from physically and biotically realistic experimental systems.

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FIGURES

Figure 1. Experimental Microcosm

Figure 2. Temporal dynamics of DEHP in the water column subsystem during the winter and summer seasons

Figure 3. Temporal dynamics of a major breakdown product of DEHP, $^{14}CO_2$ in the water column subsystem exposed to 1, 10 and 100 ug DEHP/l during the winter and summer seasons.

Figure 1

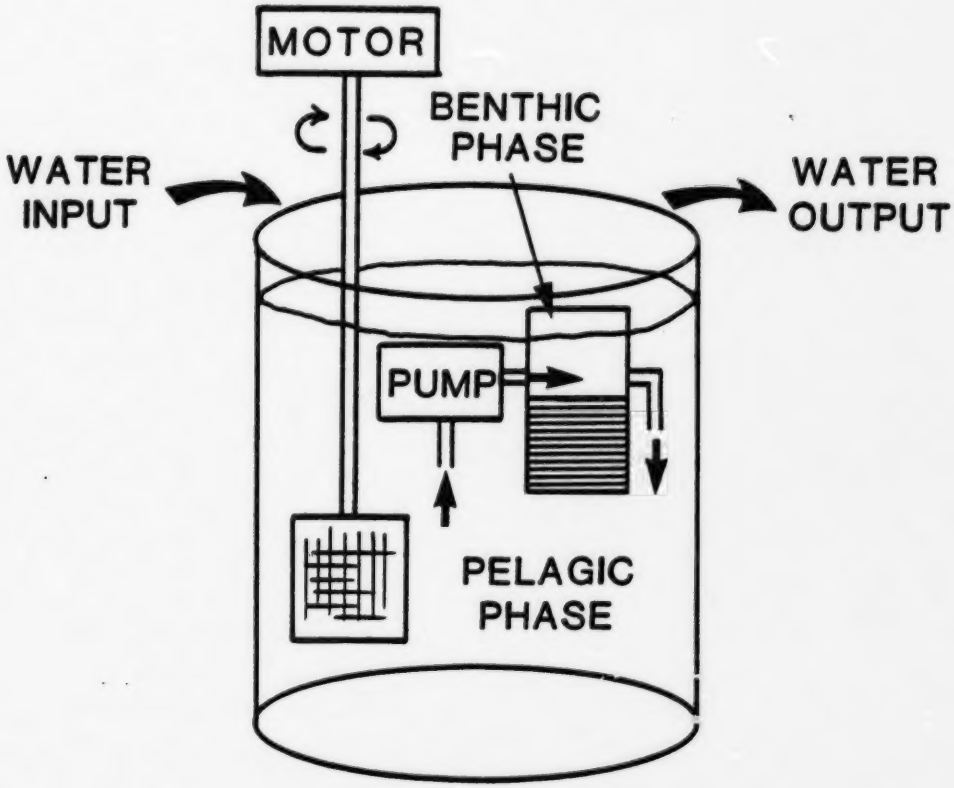
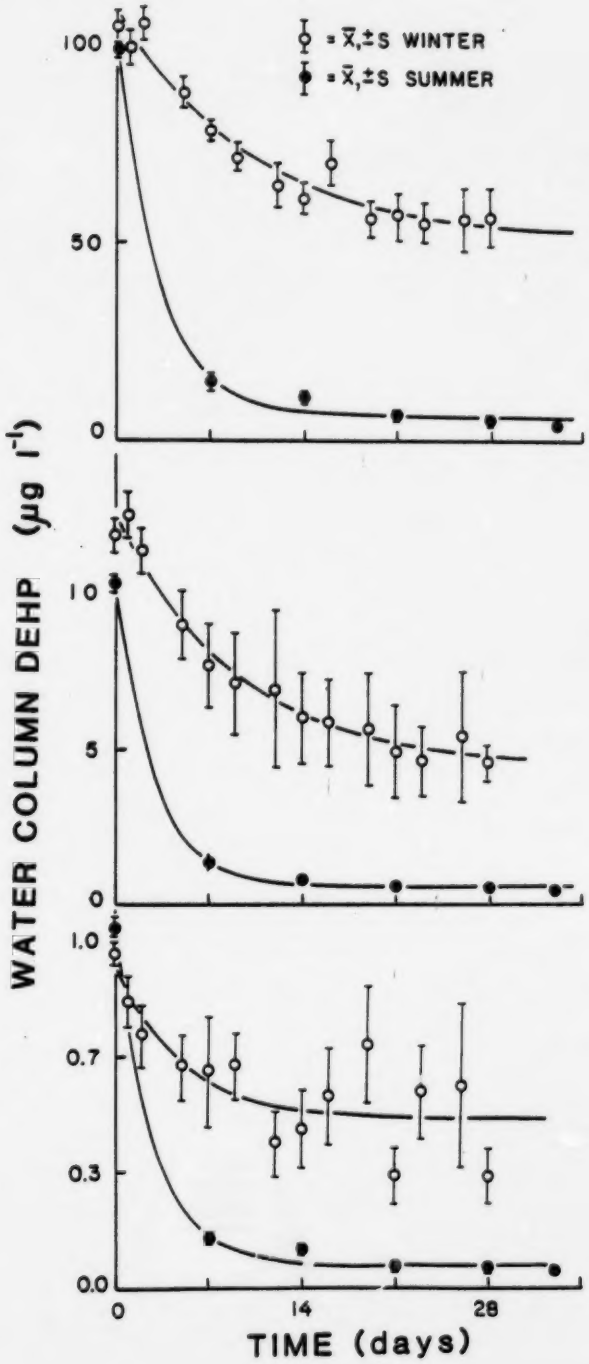

MOTOR
BENTHIC PHASE
WATER INPUT
WATER OUTPUT
PUMP
PELAGIC PHASE

Figure 2


= $\bar{X}$, ± S WINTER
= $\bar{X}$, ± S SUMMER
WATER COLUMN DEHP (µg l^{-1})
100
50
0
10
5
0
1.0
0.7
0.3
0.0
0
14
28
TIME (days)

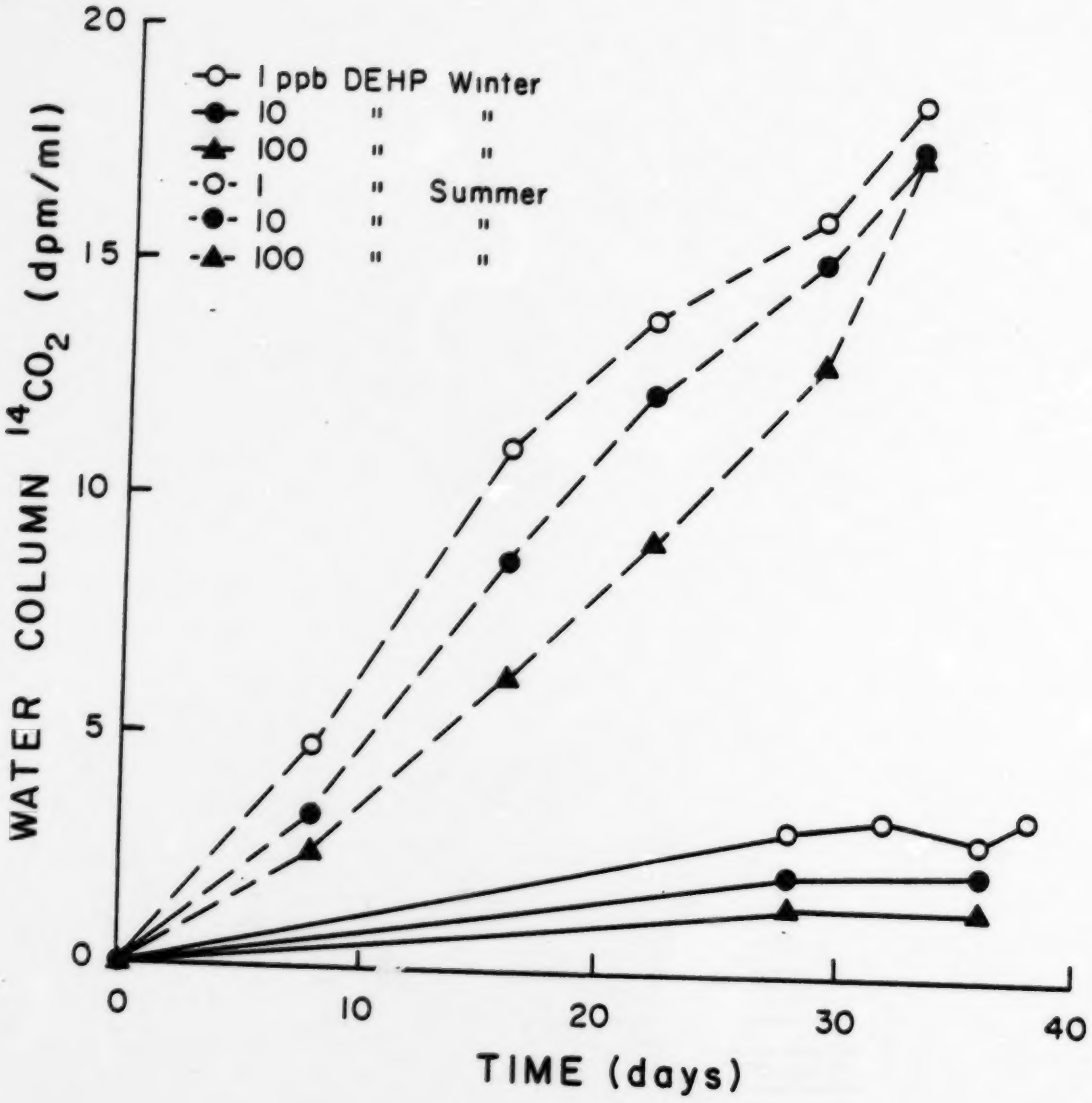

1 ppb DEHP Winter
10 " "
100 " "
1 " Summer
10 " "
100 " "
WATER COLUMN $^{14}CO_2$ (dpm/ml)
TIME (days)
0
5
10
15
20
0
10
20
30
40

Figure 3

TABLE 1 COMPARISONS OF MEAN CONCENTRATIONS OF DEHP (Log_{10}) IN THE WATER COLUMN ($\mu g\ l^{-1}$), SEDIMENT (μg (kg DRY)$^{-1}$), BENTHIC SPECIES (μg (kg WET TISSUE)$^{-1}$) AND A WATER COLUMN GENUS (μg (kg WET TISSUE)$^{-1}$) DURING THE WINTER(W) AND SUMMER (S) SEASONS.

INITIAL WATER COLUMN [DEHP]

	SEASON	1	10	100	$\bar{X}$*
VARIABLE WATER COLUMN †	W	-0.24	0.77	1.77	0.77
	S	-0.73	0.07	1.19	0.18
	$\bar{X}$*	-0.49	0.42	1.48	

	SEASON	1	10	100	$\bar{X}$*
VARIABLE SEDIMENT	W	1.61	2.36	3.68	2.55
	S	1.63	2.34	3.79	2.59
	$\bar{X}$*	1.62	2.35	3.72	

					$\bar{X}$*
Pitar morrhuana	W	2.90	3.79	4.41	3.50
	S	2.32	2.63	—	2.47
	$\bar{X}$*	2.61	3.01	4.41	

					$\bar{X}$*
Nucula annulata	W	1.86	3.01	3.77	2.88
	S	2.55	3.33	4.49	3.45
	$\bar{X}$*	2.12	3.13	4.04	

					$\bar{X}$*
Mulinia lateralis	W	3.15	4.26	5.36	4.17
	S	2.24	3.14	4.71	3.14
	$\bar{X}$*	2.76	3.94	4.71	

					$\bar{X}$*
Nephys incisa	W	1.53	2.44	3.24	2.34
	S	2.32	2.93	3.93	3.06
	$\bar{X}$*	1.83	2.62	3.54	

					$\bar{X}$*
Acartia sp.	W	3.17	2.97	4.62	3.61
	S	3.00	3.72	4.49	3.84
	$\bar{X}$*	3.14	2.82	5.04	

* BARS NEXT TO ROW AND COLUMN MEANS INDICATE SET OF MEANS NOT SIGNIFICANTLY ($\alpha = 0.05$) DIFFERENT FROM ONE ANOTHER

† MEAN CONCENTRATION OVER A THIRTY DAY PERIOD

TABLE 2 COMPARISONS OF MEAN RESPONSES OF VARIABLES IN THE WATER COLUMN AND BENTHIC SUBSYSTEMS OF MICROCOSMS EXPOSED TO AMBIENT (C) 1, 10, 100 $\mu g\ l^{-1}$ DEHP DURING THE WINTER(W) AND SUMMER(S) SEASONS.

PELAGIC SUBSYSTEM

NH_3 (μg at $\cdot l^{-1}$)

	C	1	10	100	$\bar{X}$*
W	0.39	0.43	0.36	0.37	0.39
S	0.39	0.44	0.38	0.40	0.40
$\bar{X}$*	0.39	0.44	0.37	0.39	

BACTERIA $\times 10^5$ (numbers ml^{-1})

	C	1	10	100	$\bar{X}$*
W	3.4	3.1	3.2	3.1	3.2
S	2.1	2.2	2.4	2.4	2.3
$\bar{X}$*	2.8	2.7	2.8	2.8	

ALGAE $\times 10^3$ (numbers ml^{-1})

	C	1	10	100	$\bar{X}$*
W	5.1	4.7	4.3	4.5	4.7
S	2.4	3.0	2.8	2.6	2.7
$\bar{X}$*	3.8	3.9	3.6	3.6	

TOTAL GRAZERS (number l^{-1})

	C	1	10	100	$\bar{X}$*
W	24.2	20.5	22.3	20.0	21.8
S	59.2	50.2	56.8	55.2	55.4
$\bar{X}$*	41.7	35.4	39.6	37.6	

BENTHIC SUBSYSTEM

NH_3 FLUX (μg at $\cdot m^{-2} hr^{-1}$)

	C	1	10	100	$\bar{X}$*
W	8.8	7.1	4.6	5.3	6.5
S	28.8	33.1	28.7	19.1	27.4
$\bar{X}$*	18.8	20.1	16.7	12.2	

BENTHIC DENSITY $\times 10^4$ (numbers m^{-2})

	C	1	10	100	$\bar{X}$*
W	4.3	3.3	3.7	3.3	3.47
S	3.8	2.9	4.0	3.2	3.71
$\bar{X}$*	4.04	3.06	3.93	3.22	

* HORIZONTAL AND VERTICAL LINES CONNECTING COLUMN & ROW MEANS, RESPECTIVELY, INDICATE GROUPS OF MEANS NOT SIGNIFICANTLY ($\alpha = 0.05$) DIFFERENT FROM ONE ANOTHER